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Studies on Medicinal Chemistry Potentials of Hannoa undulata Essential Oil

Anayo Joseph Uraku^{1,2}, Oluchi Helen Uraku¹, Onyema Simon Azi³, Lilian Nwanneka Ebenyi⁴, Victor Ugadu-Omoha Nwankwo⁴, Bashiru Mohamed Koroma⁵

¹Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria.
 ²Department of Biological Sciences, Njala University, Freetown, Sierra Leone.
 ³Department of Medical Laboratory, Ebonyi State University, Abakaliki, Ebonyi State
 ⁴Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria.
 ⁵Department of Chemistry, Njala University, Freetown, Sierra Leone

ARTICLE INFO	ABSTRACT
Article history: Received 21 July 2022 Revised 2 August 2022 Accepted 2 Sept 2022 Online 30 October 2022 Published	 Background: <i>Hannoa undulata</i> is a woody tree and shrub that belong to the family of Simaroubaceae. It is a perennial plant distributed in tropical and subtropical region of Africa. The plant is known for its medicinal value and the potency according to tradomedicine practitioners is very tremendous. The plant is acclaimed to be use in treatment of verse array of ailments viz: cancer, leprosy, tuberculosis, insanity, dementia, viral infection and prevention of abscesses in children among others. Due to the acclaimed therapeutic uses of the plant, this study was aimed at determining the possible bioactive components of leaves and barks of <i>H. undulata</i> using GC-MS analysis. Methods: The fresh leaves and barks of <i>H. undulata</i> were obtained from the plant and shade dried at room temperature. The dried leaves and bark stems were minced into fine powder. The bioactive combinations were done by GC-MS. Results: The mass spectrum of the compounds found in the essential oils were matched with the National Institute of Standards and Technology (NIST) library Nine and twenty one phytocompounds were identified in the methanol leaf and bark stem extracts of the plant respectively. The major chemical constituents of the leaf include; 3,3-dimethoxyprop-1-en-1-ylium (4.77%), 5-methyl-1,3-oxazolidin-2-one (6.23%), furan-2-carboxylic acid (8.16%), ethyl palmitate (8.89%), 9,12,15-octadecatrien-1-ol (12.44%), hexadecanoic acid (17.86%), 7, 10,13-octadecatrien-1-ol (36.89%) while that of the bark are hexadec-14-en-1-ylium (6.69%), oleic acid (9-octadecenoic acid) (8.17%), hexadec-15-en-1-ylium (9.12%), di-n-octyl phthalate (16.14%), 3-(hydroxy {2-[(2-methylpropoxy) carbonyl] phenyl} methoxy)-2-methylprop-1-ylium (16.51%). Conclusion: The presence of these compounds in the plant extract may be conscientious for the pharmacological properties of <i>H. undulata</i> and thus recommended as plant of phytopharmaceutical importance.

1. Introduction

Plants have been acknowledged to play central role in sustenance of man over the years. Classically, plants are used as food, ornaments and for therapeutic rationale in the treatment of miscellaneous diseases by man¹. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can result to the development of novel

and safe medicinal agents². Medicinal plants are chief source of drugs in the pharmaceutical industry when aptly synthesized and are composed of some certain organic compounds called phytochemicals which produce definite physiological actions in the human body³.

Africa hosts untapped medicinal plants whose bioactive principles have not been characterized and identified. *Hannoa undulata* is a fast-growing plant that grows up to 6-8 meters tall⁴. It belongs to the family simaroubaceae and is

locally known as Inyima Olori in the Abakaliki dialect of Ebonyi State and Gbur in the Tiv dialect of Benue State⁵. In different parts of Nigeria, it is acclaimed to be used as a cure for various ailments. *H. undulata* can be used as anti-inflammatory, antispasmodic, anti-cancer and analgesic agent⁶. The roots bark is used for treatment of stomachache while the bark and leaves are *used for* jaundice and malaria⁴. Quassinoid fractions extracted from the seeds *of H. undulate* have been reported to prevent the penetration of juveniles of *meloidogyne javanicainto* tomato roots in the soil water⁷.

The aim of this study is to divulge the bioactive compounds of methanol leaf and bark stem extracts of *H. undulata* essential oil. The outcome of this study will abet to close up slit on GC/MS appraisal of *H. undulata*.

2. Materials and methods

2.1 Materials

Methanol (Sigma Aldrich, Germany), Gas Chromatography-Mass Spectrometry (GC-MS) (Model: QP2010 PLUS Shimadzu, Japan}, mortar and pestle (Ayukalp UAP Pharma Pvt Ltd, India),

2.1.1 Collection and Identification of Plant Material

Fresh leaves and bark stems of *H. undulata* were collected from Amagu community is located at *Latitude*: 5°58'21"N. *Longitude*: 8°08'10"E in Ikwo LGA of *Ebonyi State*, Nigeria on March, 2021 in the morning between 10-11am. The plant was identified by Prof. (Mrs) Nwosu of the Department of Biological Science, Ebonyi State University, Abakaliki, Nigeria.

2.2 Preparation of plant material

The leaves (500g) of *H. undulata* were cleaned, sliced and shade dried at room temperature ($28\pm3^{\circ}$ C). The dried leaves were pulverized into fine powder using electric blender (CORONA-REF. 121, Landers and Qlink blender, Model No. OBL-15L40) while that of the bark stems were cut into small sizes and dried under the sun. The dried bark stems were pulverized using Mortar and Pestle. The powdered materials were stored in air tight polyethene bags protected from direct sunlight until required for analysis.

2.3 Plant sample extraction

Twenty (20) grams of the powdered leaves and bark stems were extracted with 50 mL of 40% methanol overnight each in a stopped bottle and with occasional stirring at room temperature (28±3°C). The samples were first sieved using muslin cloth and then filtered using Whatman No.1 filter paper. This process was repeated three times. The filtrates were concentrated under reduced pressure at 40°C for 45 min in a rotary vacuum evaporator, and then lyophilized to get a brown aromatic solid extract. The yields of the extracts were expressed in terms of the percentage of the dry weight of initial plant material used (yield 35.37% w/w). The dry extracts obtained were kept in a refrigerator at 4°C until required for use.

2.4 Column Fractionation of methanol extract of leaves and bark of *H. undulata*

The dry crude extracts were subjected to column chromatography according to standard method. The samples for the column were prepared by adsorbing 20 g of the methanol extracts of *H. undulata* with 60 g of silica gel G (60-120 mesh). The mixture was air dried and carefully layered on top of the packed silica gel in the column (14 cm length) using a glass funnel. The extracts in the column were eluted with 100 ml of methanol at the rate of 1 ml/min. The elutes were concentrated and labeled. The percentage yields of the fraction were recorded. The methanol fractions of *H. undulata* was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) of leaves and bark of *H. undulata*

GC-MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising a AOC-20i auto-sampler and chromatograph interfaced to a mass spectrometer (GC-MS). The instrument was equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The temperatures employed were; column oven temperature 80°C, Injection Temp 250°C at a pressure of 108.0 kPa, with total flow and column flow of 6.20 ml/min and 1.58 ml/min respectively. The linear velocity was 46.3 cm/sec and a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200.00°C and 250.00°C respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 µl/sec, scan range 40-800u and an injection volume of 1 µl of the plant extracts (split ratio 10:1). The total running time of GC-MS was 30 min. The relative percentage of the extracts was expressed as percentage with peak area normalization

2.6 Identification of phytocompounds

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST)⁸ having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST Ver. 3.2 library. The compound bioactivity prediction was based on Dr. Duke's Phytochemical and Ethnobotanical Databases⁹. The relative percentage amount of each phytocomponent was calculated by comparing its average peak area to the total area. The name, molecular weight, and structure of the components of the test materials were ascertained.

3. Results

The Gas chromatogram of essential oils from leaves and bark of *H. undulate*.

The results of Gas chromatogram and mass spectra of leaves and bark stems essential oil of *H. undulata* methanol are presented in Figure 1 and 2 below and showed that the leaves and bark stems stems essential oil of *H. undulata* contained nine (9) and twenty one (21) peaks respectively.

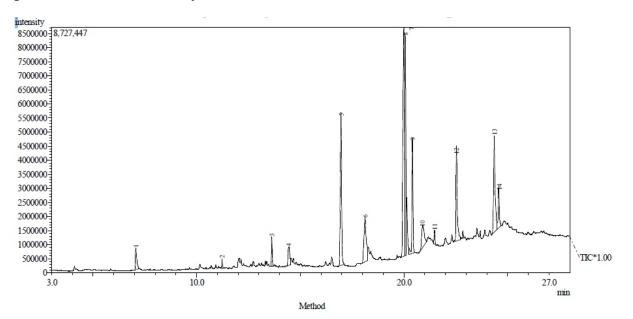


Figure 1: Gas chromatogram of essential oils of H. undulata leaves

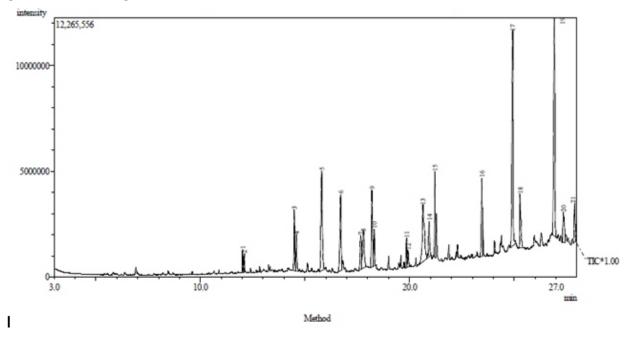


Figure 2: Gas chromatogram of essential oils of H. undulata bark stems

The proposed compounds of the leaves and bark of *H. undulate* with their peak area percentage; molecular weight and molecular formula are shown in table 1 and 2

The results indicated that the leaves had three phytochemicals with highest peak area percentage as 7, 10, 13-Octadecatrien-1-ol, Hexadecanoic acid, 9, 12, 15-Octadecatrien-1-ol with 36.89, 17.86 and 12.44 respectively while the bark stem had 3-(hydroxy {2-[(2-methylpropoxy) carbonyl]phenyl}methoxy)-2-methylprop-1-yli, um, Di-n-octyl phthalate, Hexadec-15-en-1-ylium and Oleic Acid (9-Octadecenoic acid) with 16.51, 16.14, 9.12 and 8.17 respectively.

 Table 1: Proposed phytochemicals of essential oil of *H. undulata* Leaves with retention time, peak area (%), molecular weight and formula

S/No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular
1.	m-Dihydroxy benzene	12.488	1.95	110.11	$C_6H_6O_2$
2	Furan-2-carboxylic acid	15.682	8.16	112.08	$C_5H_4O_3$
3	Niacin (nicotinic acid)	16.440	2.81	123.10	$C_6H_5NO_2$
4	3,3-dimethoxyprop-1-en-1- ylium	18.181	4.77	101.12	$C_5H_9O_2$
5	Hexadecanoic acid (Palmitic acid)	19.609	17.86	256.42	$C_{16}H_{32}O_2$
6	9,12,15-Octadecatrien-1-ol	21.217	12.44	264.44	C ₁₈ H ₃₂ O
7	5-methyl-1,3-oxazolidin-2- one	21.539	6.23	101.10	$C_4H_7NO_2$
8	7,10,13-Octadecatrien-1-ol	22.319	36.89	264.44	C ₁₈ H ₃₂ O
9	Ethyl palmitate	22.546	8.89	284.47	$C_{18}H_{36}O_2$

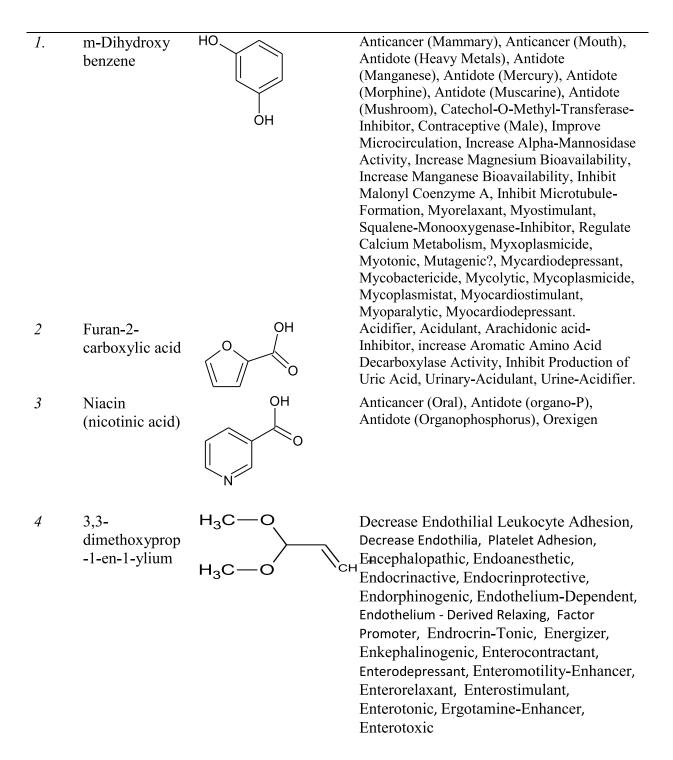
Table 2: Proposed phytochemicals of essential oil of *H. undulata* bark stems with retention time, peak area (%), molecular weight and formular

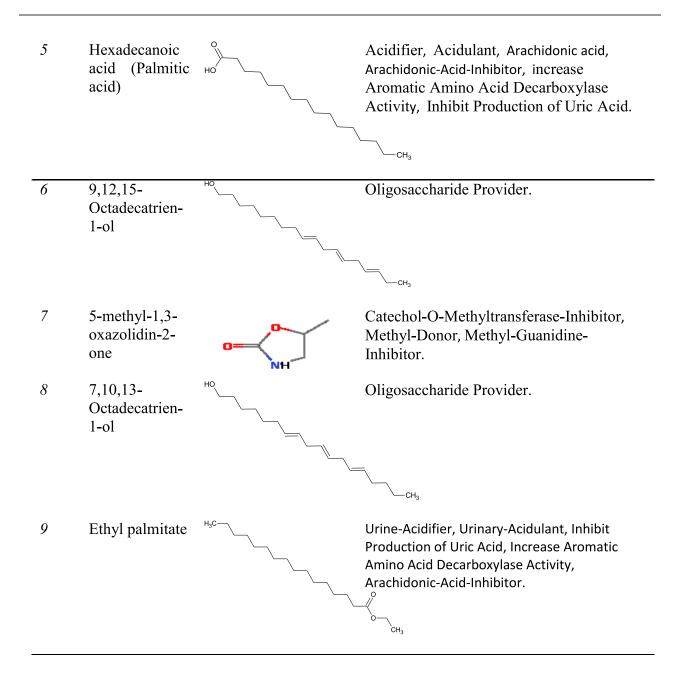
S/No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular
1	2-Ethyl-2-hexen-1-al	12.014	0.95	126.19	C ₈ H ₁₄ O
2	Undeca-6,8,10-trien-1-ylium	12.100	0.69	149.25	$C_{11}H_{17}$
3	1-Hexadecene	14.493	3.20	224.42	$C_{16}H_{32}$
4	9-Hexadecenoic acid	14.593	1.90	254.40	$C_{16}H_{30}O_2$
5	Hexadec-15-en-1-ylium	15.801	9.12	223.41	$C_{16}H_{31}$
6	Hexadec-14-en-1-ylium	6.707	6.69	223.41	$C_{16}H_{31}$
7	1,2-Benzenedicarboxylic acid, bis(2	- 17.667	2.60	278.34	$C_{16}H_{22}O_4$
	methylpropyl) ester				
8	Hexadecanoic acid	17.792	4.00	256.42	$C_{16}H_{32}O_2$
9	1-Octadecene	18.204	4.93	252.47	$C_{18}H_{36}$
10	2-Methylnonadecane	18.314	2.03	282.54	$C_{20}H_{42}$
11	9,12-Octadecadienoic acid, methyl ester	19.850	1.56	294.47	$C_{19}H_{34}O_2$
	(Methyl linolelaidate)				
12	9,12,15-Octadecatrien-1-ol	19.936	0.86	264.44	$C_{18}H_{32}O$
S/No	Name of Molecular Compounds	structu res	Bioa	ctivities	
13	Oleic Acid (9-Octadecenoic acid)	20.648	8.17	282.46	$C_{18}H_{34}O_2$
14	Octadecanoic acid (Stearic acid)	20.939	2.47	284.47	$C_{18}H_{36}O_2$
15	Cycloeicosane	21.214	4.32	280.53	$C_{20}H_{40}$
16	Nonadeca-12,14,16,18-tetraen-1-ylium	23.448	4.33	259.44	$C_{19}H_{31}$
17	Di-n-octyl phthalate	24.930	16.14	390.55	$C_{24}H_{38}O_4$
18	5-Eicosene	25.286	3.76	280.53	$C_{20}H_{40}$
19	3-(hydroxy {2-[(2-methylpropoxy)	26.923	16.51	279.35	C ₁₆ H ₂₃ O ₄
	carbonyl]phenyl}methoxy)-2-methylprop- yli, um	1-			
20	Docos-1,3-diene	27.355	3.09	306.56	$C_{22}H_{42}$
21	1-Tetraco sanol	27.894	2.68	354.65	$C_{24}H_{50}O$

Proposed phytochemicals of essential oil of *H. undulata* leaves and bark stems with their Molecular structures and Bioactivities.

The results showed that 10 compounds of the bark stem had well known biological activities while that of the leaves; all the compounds present had known biological activities

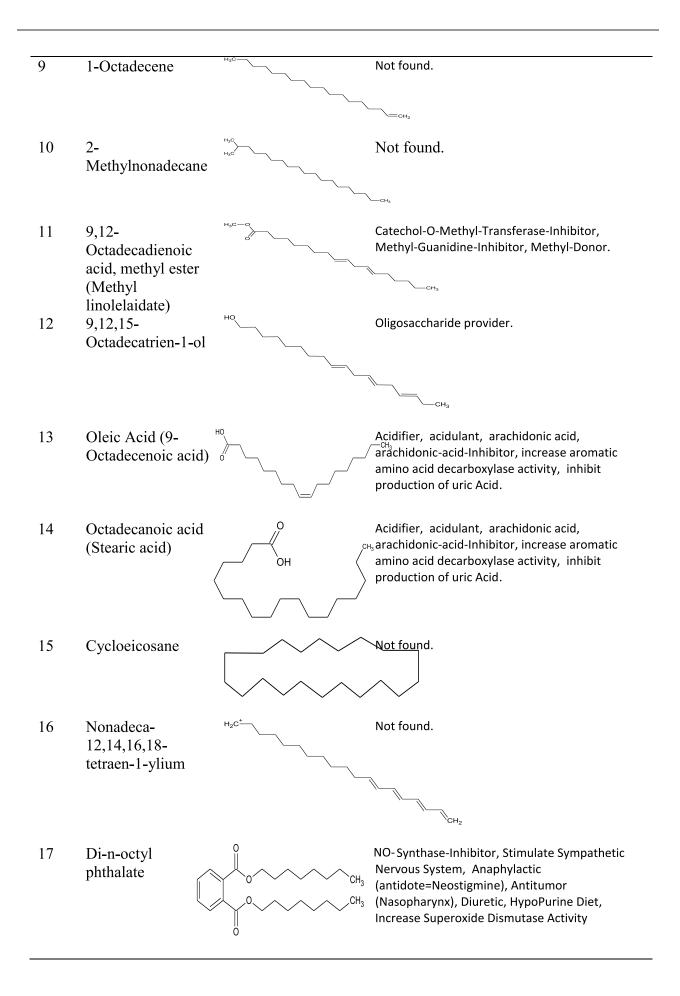
Table 3: Proposed phytochemicals of essential oil of *H. undulata* Leaves and with their Molecular structures and Bioactivities

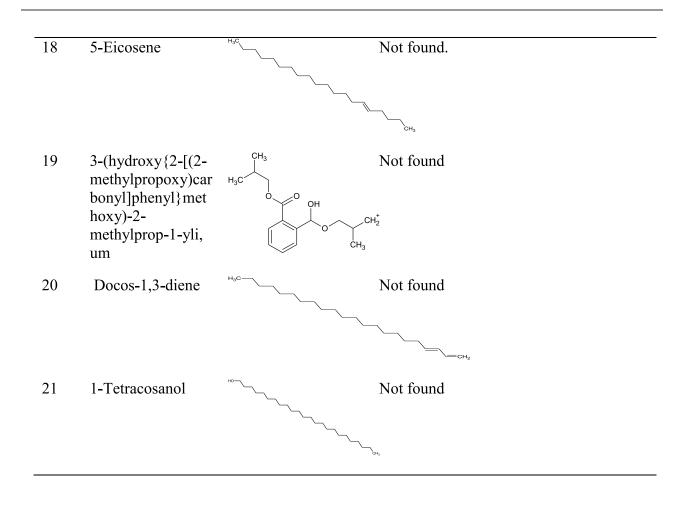




S/No	Name of Compound	Molecular structure	Bioactivity
1	2-Ethyl-2-hexen-1- al	CH	Not found I ₃
2	Undeca-6,8,10- trien-1-ylium	H ₃ C ⁻	Not found
3	1-Hexadecene	H ₂ C=	Not found
			СН3
4	9-Hexadecenoic acid	но	Acidifier, acidulant, arachidonic acid, arachidonic-acid-Inhibitor, increase aromatic amino acid decarboxylase activity, inhibit production of uric Acid.
5	Hexadec-15-en-1- ylium	H ₂ C=	сн ₃ Decrease Endothilial Leukocyte Adhesion, Decrease Endothilial Platelet Adhesion, Encephalopathic.
6	Hexadec-14-en-1- ylium	H ₃ C-////////////////////////////////////	Сн ² Endoanesthetic, Endocrinactive, Endocrinprotective, Energizer.
			—cH₂́
7	1,2- Benzenedicarboxyl ic acid, bis(2- methylpropyl) ester	H ₃ C O O O O O O	Acidifier, acidulant, arachidonic acid, arachidonic-acid-Inhibitor, increase aromatic amino acid decarboxylase activity, inhibit production of uric Acid. CH ₃
8	Hexadecanoic acid	но	Acidifier, acidulant, arachidonic acid, arachidonic-acid-Inhibitor, increase aromatic amino acid decarboxylase activity, inhibit production of uric Acid. —

Table 4: Proposed phytochemicals of essential oil of *H. undulata* bark stems with their Molecular structures and Bioactivities





4. Discussion

Gas chromatogram and mass spectra of leaves and bark stems essential oil of H. undulata methanol are presented in Figures 1 and 2 which showed nine and twenty-one peaks respectively. These suggest presence of nine and twentyone compounds which implies that the bark contained more of the phytocompunds than the leaves. The proposed compounds, retention time (RT), peak area percentage, molecular weight and molecular formula are discussed in table 1 and 2. The phytochemicals with highest peak area percentage in the leaves are 7, 10, 13-Octadecatrien-1-ol, Hexadecanoic acid, 9, 12, 15-Octadecatrien-1-ol with peak area percentage of 36.89, 17.86 and 12.44 respectively while m-Dihydroxy benzene, Niacin and 3, 3dimethoxyprop-1-en-1-ylium are low in order of 1.95, 2.81 and 4.77 respectively but in the bark stem, phytochemicals with highest peak area percentage are 3-(hydroxy{2-[(2methylpropoxy) carbonyl]phenyl}methoxy)-2methylprop-1-yli, um, Di-n-octyl phthalate, Hexadec-15en-1-ylium and Oleic Acid (9-Octadecenoic acid) with 16.51, 16.14, 9.12 and 8.17 respectively but 2-Ethyl-2hexen-1-al, Undeca-6,8,10-trien-1-ylium, 9,12,15-Octadecatrien-1-ol and 9,12-Octadecadienoic acid, methyl ester (Methyl linolelaidate) are less than 2. Amongst the compounds present, only hexadecanoic acid (palmitic acid) and 9, 12, 15-octadecatrien-1-ol was common in both leaves and bark stem while others are variable with different amounts (percentage peak areas).

Table 3 and 4 showed the nature and structures of the phytocompounds with their bioactivities found in the essential oil of the leaves and barks of the plant. Out of 21 compounds found in the bark stem essential oil, only 10 compounds have known biological activities while in that of the leaf, all have known biological activities. Based on studies, some of the constituents revealed by GC–MS are biologically active compounds. They were proven to possess pharmacologic activities which may contribute to the healing potential of the plant.

According to the report of Savithramma *et al.*⁶ *Hannoa undulate* can be used as anti-inflammatory, antispasmodic,

anti-cancer and analgesic agent. This is attributed to the presence of m- dihydroxy benzene, niacin (nicotinic acid) and di-n-octyl phthalate in the plant.

5-methyl-1, 3-oxazolidin-2-one and 9, 12 octadecadienoic acid, methyl ester (methyl linolelaidate) has been proven to be essential in treatment of chronic infection like Parkinson's disease. This potential could be attributed to inhibition of catechol-O-methyltransferase. Catechol-O-methyltransferase is involved in degradation of neurotransmitters, but the inhibitors oppose the degradation of neurotransmitters¹⁰.

Furan-2-carboxylic acid, hexadecanoic acid (palmitic acid), ethyl palmitate, octadecanoic acid (stearic acid), 9-O c t a d e c e n o i c a c i d (o l e i c a c i d) and 1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester have been proven to increase aromatic amino acid decarboxylase activity that enhances the production of several neurotransmitters like the serotonin and dopamine^{11,12} Also, they have been reported to be an acidifier, acidulant and arachidonic acid inhibitor¹³. Acidifiers reduce the pH of the body and as well important in food digestion especially in patients suffering from achlorhydria. Interestingly, urinary acidifiers maintain low urine pH when mingled with diet and thus helps in eliminating urinary tract infections, dissolving alkaline bladder stones and promoting kidney health.

9, 12, 15-Octadecatrien-1-ol, 7, 10, 13-Octadecatrien-1-ol and 9, 12, 15-Octadecatrien-1-ol are provider of oligosaccharides which in the cells produce specific carbohydrate-binding ligands; lectins which mediate celladhesion with oligosaccharides¹⁴. Selectins a family of lectins mediate certain cell to cell adhesion processes, like those of the leukocytes to endothelial cells, thus allowing the white blood cells to help in eliminating the infection or damage¹³. The breakdown of glucose to pyruvate for production of ATP and energy is enhances by glycolytic activity¹⁵. The oligosaccharide provider in H. undulata could enhance ATP production as well as inhibition of uric acid production. This inhibition could help in treatment of gout, diabetes, and kidney stones. It is worthy to note that purine nucleotides break down leads to formation of uric acid and its high amounts in blood can lead to gout, diabetes, and formation of ammonium acid urate (kidney stones)¹⁰. Octadecanoic acid (stearic acid), 9-Octadecenoic acid (oleic acid), hexadecanoic acid (palmitic acid), 1, 2-Benzene dicarboxylic acid, bis (2-methylpropyl) ester and ethyl palmitate identified in H. undulata oils may help in the inhibition of uric acid.

Di-n-octyl phthalate as an inhibitor of nitric oxide synthase could be of pharmacological importance in Erectile dysfunction (ED) management and thus enhances sexual desire and pleasure The main mediator of penile erection is nitric oxide (NO), which is produced through the activity of nitric oxide synthase (NOS) expressed in endothelial and neuronal cells^{16,17}. The presence of 3, 3-dimethoxyprop-1en-1-ylium in the plant might decrease adhesion of leukocytes, Endothilia and Platelet to endothelial cells, thus allowing the white blood cells to help in eliminating the infection or damage. Similarly, this phytocompound acts as energizer, Encephalopathic, Endoanesthetic, Endocrinactive, Endocrinprotective, Endorphinogenic, Endothelium-Dependent, Endothelium-Derived Relaxing, Factor Promoter, Endrocrin-Tonic, Enkephalinogenic, Enterocontractant, Enterodepressant, Enteromotility-Enhancer, Enterorelaxant, Enterostimulant, Enterotonic, Ergotamine-Enhancer and Enterotoxic.

5. Conclusions

This is the first report on the GC-MS analysis of essential oil of *H. undulate leaves and bark* stems. The bioactive compounds identified by GC-MS analysis of the leaves and bark stem have demonstrated to be helpful in amelioration of many diseases and therefore invaluable in pharmaceuticals as well as in health care services. Further studies are needed to isolate pure active principle of the extract as well as to elucidate their exact mechanism of action in various diseases.

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